Recommendations and Resources For Teaching Metabolic Acidosis To The Undergraduate Student In Exercise Physiology

Robert A. Robergs, Ph.D., FASEP, EPC
Center For Exercise and Applied Human Physiology, Exercise Science Program, University of New Mexico, Albuquerque, NM

Address for correspondence: Robert A. Robergs, Ph.D., FASEP, EPC, Center For Exercise and Applied Human Physiology, Exercise Science Program, Department of Physical Performance and Development, Johnson Center, Room B143, University of New Mexico, Albuquerque, NM, 87131-1258; Phone: (505) 277-2658; FAX (505) 277-9742, email: rrobergs@unm.edu

Introduction

During the last two years it has become obvious to me that the textbook content we use to teach the cause of metabolic acidosis in exercise physiology is incorrect. This realization led to the content of presentations that myself or my Ph.D. students have given in the last two ASEP national meetings. In addition, recent interactions with Will Hopkins on this topic motivated me to write the manuscript that was recently published in Sportscience [www.sportsci.org/jour/0102/rar.htm](http://www.sportsci.org/jour/0102/rar.htm) (1).

Based on feedback from colleagues via email, and from the recent ASEP national meeting in Memphis, there is understandable concern over how to teach the “correct” explanation of exercise-induced acidosis without relying on advanced biochemistry and organic chemistry. Consequently, the purpose of this manuscript is to provide recommendations on how to teach exercise-induced metabolic acidosis. In this manuscript I provide figure resources that can be used to supplement/correct textbook material. I also provide figures and explanations that differ in complexity so that you can differentiate lecture material between entry-level students, the more advanced undergraduate student, and graduate students. You can use these figures by copying and saving them for use in your own lecture material. In addition, at the end of the manuscript are links to all the
components of the figures, thereby providing you with the flexibility to use only the more basic versions for lower level undergraduates, and more complex versions for more advanced students.

**The Current Explanation of Metabolic Acidosis**

As textbook material presents an explanation of acidosis that is incorrect and obviously not based on biochemical fact, it is important to first inform the students of this fact. After stating this, I explain that the textbook approach to this topic has a long history in pure and applied physiology and biochemistry, and can be dated back to the 18th century when lactic acid was first discovered (2). During the early 20th century, added research showed that lactic acid was produced during conditions of low oxygen content (hypoxia), and that muscle contraction also became impaired (3). This research led to the assumption that lactic acid production caused decreases in cellular and blood pH, which in turn caused the symptoms of muscle and body fatigue during intense exercise. This evidence was not cause-and-affect, but rather guilt by association. Lactic acid was shown to increase when pH decreased, and fatigue followed. No evidence existed that proved that the acidic form of lactate (lactic acid) was produced, or that protons released from lactic acid caused the acidosis. At this time there was no knowledge of how acids and bases interacted chemically, and therefore, no reason to distinguish between an acid and an acid salt (Figure 1).

![Figure 1: Structural differences between a carboxylic acid and the salt of the acid (when there is no proton on the acid functional group).](image)

It was not until the work of Henderson and Hasselbach that chemists realized that acids release protons, that the proton concentration quantified acidosis, that the propensity for the release of a proton from an acid was specific to a given acid, and that these qualities can be quantified. Based on the work of Henderson and Hasselbach, we now refer to the pH at which an acid releases protons, equivalent to a proton release from half
of the molecules, as the pK. The lower the pK, the greater the release of protons for a given pH above the pK, and the stronger the acid. For example, an acid with a pK of 3 exposed to a cellular pH of 7 will have protons released from almost all of the acid molecules. If these protons are not buffered or removed from the cell, cell pH will decrease (increased [H⁺]), causing acidosis. Figure 1 illustrates the proton release from an acid, and differentiates between an acid and an acid that has lost the proton (acid salt).

The current explanation of exercise-induced acidosis is based on the production of lactic acid, which, due to a low pK (3.86), immediately dissociates to lactate (La⁻) and a proton (H⁺), as depicted in Figure 2. To maintain charge neutrality, the lactate ion interacts with a positively charged ion (cation); typically sodium forming sodium lactate (Na⁺La⁻). This interpretation results in the logical belief that in-vivo the net result from the production of lactic acid is sodium lactate and the release of a proton. A generic chemical equation used to support this explanation is presented in Figure 3, along with extensions of this biochemistry to bicarbonate buffering, and stimulation to ventilation causing the ventilation threshold to coincide reasonably close to the lactate threshold.

![Figure 2: The theoretical ionization of lactic acid to sodium lactate](image)

![Figure 3: The theoretical biochemical connections between lactic acid production, bicarbonate buffering, the generation of non-metabolic CO₂, and increased chemical (H⁺ and CO₂) stimulation to ventilation.](image)
Once this content is presented, it must be reiterated that there is no scientific evidence for explaining acidosis by the production of lactate. Furthermore, it is important to express that several scientists have questioned the explanation of acidosis caused by lactate production (4-10), and that an alternate explanation of acidosis is therefore supported by numerous academics and scientists. In short, you are not alone in wanting to provide another, more correct, explanation of the biochemical causes of acidosis.

Finally, it should also be stressed that the assumption that acidosis is caused by the production of metabolic acids is wrong. Acids are one source of free protons, but free protons can also be released by chemical reactions. Thus, it is possible to have chemical reactions contribute to acidosis without producing any acids at all!

**Explaining the Cause of Acidosis During Exercise**

Once the more traditional explanation of acidosis is given, there obviously needs to be a convincing explanation of the true causes of acidosis. Due to the lack of any textbook material to help you do this, the content that follows is vital to your lecture material.

**Why Lactate Production Does Not Produce Protons**

I think a good place to start is to explain why it is impossible for lactate production to release a proton. This is really quite easy to do, and involves the presentation of one reaction; the phosphoglycerate kinase (PGK) reaction (Figure 4). The PGK reaction is the second reaction of phase 2 of glycolysis, and is the first “acid producing” reaction of glycolysis. I use quotation marks in this labeling, for as you can see, the product of this reaction, 3 phosphoglycerate, is produced as an acid salt and therefore is never in an acid form that releases a proton.
Figure 4: The phosphoglycerate kinase reaction of phase 2 of glycolysis. Note that the phosphate on the first carbon of 1,3 bisphosphoglycerate is not associated with a proton, and that the phosphate transfer to ADP occurs without a proton being involved.

I think it is necessary to teach even the lower-level exercise physiology student the details of the PGK reaction. It is important to communicate the fact that if 3 phosphoglycerate is formed devoid of a proton, then every other carboxylic acid intermediate of glycolysis that follows is also an acid salt. We need to expect that our students understand this. If they do, then it is common sense to extend these facts into the impossibility for lactate production to release a proton, as there is no proton to release!

The lactate dehydrogenase (LDH) reaction can then be presented in a new way (Figure 5). The LDH reaction is necessary to regenerate NAD⁺, as well as consume a proton. As such, lactate production retards, not contributes to acidosis (Figure 6).

Figure 5: The lactate dehydrogenase (LDH) reaction. Note that pyruvate is reduced by NADH plus an added proton. Lactate production consumes, not produces a proton.
Where do the protons come from?

If lactate production does not release protons and eventually cause acidosis, where do the protons come from? I recommend stating the two main sources of protons during catabolism; glycolysis and ATP hydrolysis. These sources of protons can then be explained.

**Glycolysis**

The details of the chemical reactions of glycolysis have been known for decades. Stryer (11) has summarized these best in his textbook of biochemistry. I have presented this material in table 1, and added color to identify protons consumed and produced.
Table 1: The reactions of glycolysis, including the lactate dehydrogenase (LDH) reaction and a tally for ATP and protons (H⁺).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme</th>
<th>ATP</th>
<th>H⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glycolysis Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 6 carbon intermediates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose + ATP ⇌ Glucose 6-phosphate + ADP + H⁺</td>
<td>Hexokinase</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>Glucose 6-phosphate ⇌ Fructose 6-phosphate</td>
<td>Phosphoglucone isomerase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose 6-phosphate + ATP ⇌ Fructose 1,6-bisphosphate + ADP + H⁺</td>
<td>Phosphofructokinase</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>Fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate</td>
<td>Aldolase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 3 carbon intermediates (ATP and H⁺ tally numbers are based on doubling the reactions)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dihydroxyacetone phosphate ⇌ glyceraldehyde 3-phosphate</td>
<td>Triose phosphate isomerase</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glycolysis Phase 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glyceraldehyde 3-phosphate + Pi + NAD⁺ ⇌ 1,3 bisphosphoglycerate + NADH + H⁺</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1,3 bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP</td>
<td>Phosphoglycerate kinase</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3-phosphoglycerate ⇌ 2-phosphoglycerate</td>
<td>Phosphoglyceromutase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-phosphoglycerate ⇌ Phosphoenolpyruvate + H₂O</td>
<td>Enolase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphoenolpyruvate + ADP + H⁺ ⇌ Pyruvate + ATP</td>
<td>Pyruvate kinase</td>
<td>2</td>
<td>-2</td>
</tr>
<tr>
<td><strong>ATP and H⁺ tally</strong></td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>LDH Reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate + NADH + H⁺ ⇌ Lactate + NAD⁺</td>
<td>Pyruvate kinase</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td><strong>ATP and H⁺ tally</strong></td>
<td></td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Adapted from Stryer (1988)

When evaluating the balance of the reactions of glycolysis, it is clear that glycolysis produces two protons for the production of 2 pyruvate molecules. When adding the LDH reaction, the production of two lactate molecules consumes these protons, resulting in no net proton release (Figure 7). Clearly, lactate production during intense exercise is important to retard an increasing rate of proton release due to an increased...
flux of substrate through glycolysis. However, there is still a source of protons that must contribute to acidosis.

What is this source?

\[
\text{glucose} + 2 \text{ ADP} + 2 \text{ Pi} + 2 \text{ NAD}^+ \quad \overset{\text{ATP hydrolysis}}{\longrightarrow} \quad 2 \text{ pyruvate} + 2 \text{ NADH} + 2 \text{ H}^+ + 2 \text{ ATP} + 2 \text{ H}_2\text{O}
\]

\[
2 \text{ pyruvate} + 2 \text{ NADH} + 2 \text{ H}^+ \quad \overset{\text{2 lactate} + 2 \text{ NAD}^+}{\longrightarrow} \quad 2 \text{ lactate} + 2 \text{ NAD}^+ + 2 \text{ ATP} + 2 \text{ H}_2\text{O}
\]

**Figure 7:** The net products from glucose oxidation to pyruvate and subsequent reduction to lactate. Note that lactate production consumes the proton release from glycolysis.

**ATP Hydrolysis**

The chemistry of ATP hydrolysis is crucial to understanding acidosis. Every time an ATP molecule is converted to ADP and Pi, a water molecule is needed to provide a hydroxyl group and added electron to the terminal phosphate, forming inorganic phosphate (HPO_{4}^{2-}) (Figure 8). The remaining proton is released into solution at physiological pH due to the acidic pK (6.82) of the relevant oxygen of the terminal phosphate of ADP.

**Figure 8:** a) The biochemistry of ATP hydrolysis, and b) the pK characteristics of inorganic phosphate (Pi).
However, your students’ may question the importance of this reaction, as ATP hydrolysis occurs continuously inside a cell, and during low to moderate exercise intensities there is no acidosis despite dramatically increased rates of ATP hydrolysis and minimal lactate production. Although this observation is correct, there is no net proton accumulation as protons from ATP hydrolysis are transported into the mitochondria. Furthermore, the protons from glycolysis (which are minimal during low intensity exercise due to the predominance of lipid oxidation) are involved in the shuttling of electrons from cytosolic NADH in to the mitochondria. Thus, during steady state conditions, proton balance in the cytosol is maintained by a near total dependence on mitochondrial respiration for ATP regeneration (Figure 9).

Figure 9: A summary of the exchange of protons, NADH and NAD⁺ between glycolysis and the mitochondria. Note that this depiction does not account for the double reactions of phase 2 of glycolysis, and is designed to show the proton (H⁺) interactions between the sarcolemma, cytosol and mitochondria.
As soon as non-steady state conditions prevail in contracting skeletal muscle, there is a deficiency in how mitochondria are consuming protons, as well as regenerating ATP. Thus, there is an increased need for cytosolic ATP to be regenerated from glycolysis, and a resulting accumulation of NADH and protons. Thus, acidosis coincides with non-steady state exercise conditions, which also coincide with increased lactate production due to the need to regenerate cytosolic NAD$^+$ (maintain cytosolic redox; NAD$^+$/NADH) and continue phase 2 of glycolysis.

**What About Glycogenolysis and an Increased Glycolytic Dependence on Glycogenolysis?**

During increasing exercise intensity there is an increased dependence on glucose-6-phosphate production from glycogenolysis. Glycogenolysis does not involve the release of a proton as inorganic phosphate, rather than ATP (see hexokinase reaction), is used to phosphorylate a glucose residue to glucose-1-phosphate (Figure 10). However, due to the absence of the ATP cost to phosphorylate glucose, when glycolysis is fueled by muscle glycogen there is a net of 3 ATP regenerated. Thus, during these conditions, glycolysis would yield 1 net proton to produce 2 pyruvate, 2 lactate would account for 2 protons consumed, and hydrolysis of the three ATP would result in 3 protons released. The proton balance of these events is still 2 lactate + 2 protons. Consequently, an increasing glycolytic dependence on muscle glycogen does not change the net proton and lactate release from glycolysis and associated ATP hydrolysis.

![Figure 10: The phosphorylase reaction, which reveals that no proton is released during the formation of glucose-1-phosphate.](image-url)
This is likely to be more than enough material for the lower level undergraduate student. However, added reactions that involve proton release or consumption occur during catabolism in skeletal muscle during moderate to intense exercise, and I have detailed these reactions elsewhere (1). This added biochemical information is recommended content to present to more advanced students.

**Added Material For the Advanced Student**

For advanced courses/students in exercise physiology, it is recommendable to present a more comprehensive coverage of the reactions that influence cytosolic pH. The reactions of the phosphagen system and carbohydrate catabolism that influence proton balance are presented in table 2.

| Table 2: Reactions of the phosphagen system and glycolysis that influence cellular pH. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Reaction                                      | Enzyme                                        | Capacity* |
|                                               |                                               | -‘ve | +’ve |
| **Phosphagen System**                         |                                               |      |      |
| *ATP ↔ ADP + Pi + H⁺                          | ATPase                                        | 70   |      |
| Creatine phosphate + ADP + H⁺ ↔ Creatine + ATP| Creatine Kinase                               | 20   |      |
| AMP + H⁺ ↔ IMP + NH₄                         | AMP deaminase                                 | 3    |      |
| **Glycolysis**                                |                                               |      |      |
| Glucose + ATP ↔ Glucose 6-phosphate + ADP + H⁺| Hexokinase                                    | 65   |      |
| Fructose 6-phosphate + ATP ↔ Fructose 1,6-bisphosphate + ADP + H⁺ | Phosphofructokinase | 65   |      |
| Glyceraldehyde 3-phosphate + Pi + NAD⁺ ↔ 1,3 bisphosphoglycerate + NADH + H⁺ | Glyceraldehyde 3-phosphate dehydrogenase | 130  |      |
| Phosphoenolpyruvate + ADP + H⁺ ↔ Pyruvate + ATP | Pyruvate kinase                              | 130  |      |
| **Lactate Dehydrogenase**                    |                                               |      |      |
| Pyruvate + NADH + H⁺ ↔ Lactate + NAD⁺         | Lactate Dehydrogenase                         | 30   |      |
| **Proton Tally**                             |                                               | 183  | 330  |

* Capacity is indicated as a total relative molar amount (mmol/kg wet wt) of protons based on 3 min of intense exercise to fatigue.
# An estimate of anaerobic ATP hydrolysis based on research of the accumulated oxygen deficit (12-15) These calculations do not adjusted for accumulations of pyruvate and acetyl-carnitine, or additional intermediates of carbohydrate oxidation (cytosolic or mitochondrial).
The data of table 2 reveals that research evidence indicates that muscle proton release almost doubles that of proton consumption during intense exercise. Lactate production is clearly shown to be non-stoichiometric to proton release, with glycolysis (130 mmol/kg/3 min) and then ATP hydrolysis that occurs in excess of mitochondrial respiration (70 mmol/kg/3 min) the source of protons.

I have detailed the biochemistry of the reactions of table 2 elsewhere (1), and all figures are provided in the resource appendix to this manuscript. A summary illustration of these reactions is presented in Figure 11.

![Figure 11: A schematic of the proton consuming and releasing reactions of the phosphagen system and glycolysis.](image)

When pyruvate accumulates in the cytosol or mitochondria, or carbon molecules from pyruvate accumulate in the mitochondria as acetyl groups (eg. acetyl carnitine), there is a decrease in the protons consumed by the LDH reaction or mitochondria compared to the proton release. This results in an increased net proton release. Once again, it is very important to stress that when the cellular ATP demand exceeds ATP
supply from mitochondrial respiration, glycolytic flux increases, and there is potential to exceed the capacity of the LDH reaction. When this happens, net proton release increases, the buffer capacity of the cell is eventually taxed, the capacity of lactate and proton efflux from the cell is exceeded, and cellular proton accumulation (cellular acidosis) occurs.

Unlike the textbook content on this issue, lactate production is necessary to retard the release of protons, and in fact aid the removal of protons from the cell. Therefore, lactate can be summarized to be beneficial for the following reasons;

1) Lactate production consumes protons.

2) Lactate regenerates \( \text{NAD}^+ \) for phase 2 of glycolysis, thereby enabling continued ATP regeneration from glycolysis.

3) Lactate aids the transport of protons from the cell via the monocarboxylate lactate-proton transporter, further retarding cellular acidosis.

Finally, the more advanced student should be able to grasp the concept and influence of an altered motor unit recruitment on muscle metabolism, and whole body measurements of acidosis and metabolism. It is common knowledge that another factor coincident with the onset of acidosis is an increased dependence on fast twitch motor unit recruitment (16). From a theoretical basis, this altered motor unit recruitment profile is extremely important as a contributor to indices of muscle and whole body acidosis. Fast twitch muscle has less mitochondrial density compared to slow twitch muscle, and therefore, will rely more on glycolytic flux for ATP regeneration. Having less mitochondria means that fast twitch muscle will accumulate more NADH and protons due to the low capacity for proton transport into the mitochondria. Although additional lactate production occurs in fast twitch muscle, the capacity of this consumption of protons is limited by enzyme kinetics and thermodynamics combined with the saturatable kinetics of the lactate-proton transporter system.

**Arguments in Favor of a Lactic Acidosis**

Despite the unquestionable biochemistry of metabolic acidosis, I am still challenged on such a biochemical based explanation of metabolic acidosis. These criticisms are based on one of two opinions:
1) When the cell is reliant on glycolysis, you get two lactate and 2 protons from the hydrolysis of the ATP from glycolysis. Thus, the net result is lactic acid.

Obviously the biochemistry reveals that regardless of the net result, the cause of the free proton release is not lactate production. In short, the answer is as simple as that. However, to reinforce the danger of such reductionist thinking, I state the following.

The glycolysis, lactate and ATP hydrolysis balance is only relevant when the following conditions are met; a) all pyruvate that is not completely oxidized within the mitochondria is converted to lactate, b) cellular ATP concentrations do not decrease, c) no other reactions influence cellular proton release or consumption, and d) attempts to balance proton release and consumption during intense exercise reveals a net proton release that far exceeds lactate production. There is no stiochiometry between lactate production and proton release.

We know that each of these requirements are not meet during intense exercise. Pyruvate can accumulate in the cytosol and blood, and carbons from pyruvate accumulate within the mitochondria as acetyl-carnitine. Such combined accumulation can amount to as much as 10 mmol/kg wet wt, causing an equimolar amount of added net proton release (due to the decrease in proton removal/consumption from lactate production and mitochondrial respiration). Cellular ATP decreases from approximately 8 to 5-6 mmol/kg wet wt during intense exercise to volitional fatigue (13,15).

Finally, we know that each of the creatine kinase and AMP deaminase reactions consume protons. Ironically, the net sum of the creatine phosphate converted to creatine, and the AMP converted to IMP approximates the lactate produced. This balance would theoretically, according to the reductionist argument stated above, minimize net free proton release. However, acidosis does develop. Therefore, it is no surprise to estimate that proton release far exceeds lactate production in skeletal muscle (1) (table 2), and there is no rational argument to retain a “lactic acidosis” depiction of cellular metabolic acidosis.
2) For cells such as the red blood cell, glycolysis is the only metabolic pathway that regenerates ATP, but the red blood cell does not become acidotic, or is believed to contribute to blood acidosis.

I was challenged with this response by a biochemist when in Australia during my sabbatical. I was initially stumped by this question, but 5 minutes after my talk, the answer was obvious!

The red blood cell is probably the most highly buffered cell in the body, is bathed in a buffered solution (blood plasma), and has a metabolic rate that is extremely small compared to contracting skeletal muscle. Furthermore, the buffer potential of the red blood cell and blood is “recharged” during pulmonary ventilation. In any event, the net proton release form red blood cells is so small that the red blood cell is a major buffer agent to protons released from more metabolically active cells (eg. contracting skeletal muscle). There is no substance to this argument!

I hope you find that this depiction of metabolic acidosis is helpful to you, and that you can use the recommendations and resources provided to update textbook content on this topic, thereby improving the accuracy of your lectures to your students.

References


